as well as perception consistency across individuals. An overly strong capability of pattern completion would deny an animal its ability to discriminate different odorants. Excessive plasticity would lead an animal to perceive the same object differently after an unreasonably short period. Similarly, completely random connections would make it difficult to generate consistent perception across individuals, so that fruits may no longer consistently smell "fruity" to human individuals. The connections in the piriform cortex must be carefully carved to achieve a delicate balance among different behavioral needs. The wiring stochasticism in the piriform cortex highlights the needs and challenges of searching for logic in the neural circuits underlying animals' amazing sense of smell.

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MeCP2: Phosphorylated Locally, Acting Globally

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In this issue of *Neuron*, Greenberg and colleagues revise our understanding of how activity-dependent MeCP2 phosphorylation regulates distinct aspects of brain development and circuit function. The study also suggests a prominent role for MeCP2 in the regulation of global chromatin state in vivo.

MeCP2 (X-linked methyl-CpG-binding protein 2) is an abundant nuclear protein that binds methylated DNA and historically has been thought to act as a transcriptional repressor critical for normal neural development. Mutations in the gene encoding MeCP2 cause the Autism-spectrum disorder Rett Syndrome (RTT). In this issue of *Neuron*, Cohen, Greenberg, and colleagues demonstrate that activity-induced phosphorylation of MeCP2 at a single serine residue (S421) controls distinct aspects of synapse development and social behavior (Cohen et al., 2011). In contrast to prior studies implicating this phosphorylation event in the dynamic regulation of MeCP2 binding at specific promoters, the present study suggests that the primary function of MeCP2 in neurons is not to regulate transcription of specific genes but rather to regulate chromatin remodeling on a global scale.

DNA methylation is an epigenetic modification that plays an essential role in mammalian embryogenesis presumably through repressive effects on gene transcription. Functional studies have demonstrated that methylation of DNA can inhibit transcription by either blocking transcription factor access to target regions or by acting as homing sites for methyl-CpG binding domain proteins (MBDs) (Bird, 2002). Interest in DNA methylation and nervous system development took an unprecedented turn over a decade ago when Zoghbi and colleagues first identified independent mutations in the MBD and transcriptional repression domains of the human *MECP2* gene as disease-causing mutations leading to RTT (Amir et al., 1999). Rett syndrome is a progressive and debilitating neurodevelopmental disorder that predominantly affects young girls at an estimated 1–10,000–15,000 ratio. Mice that lack MeCP2 either globally or conditionally in the central nervous system develop symptoms similar to RTT (Chen et al., 2001; Guy et al., 2001). If MeCP2 functions as a transcriptional repressor, then the identification of genes dependent upon MeCP2 for proper transcriptional regulation should provide insight into the pathophysiology of RTT. Numerous groups attempted to answer this question by examining global transcriptional profiles from forebrain, hypothalamus, or

cerebellum of MeCP2-deficient mice using oligonucleotide technology. Surprisingly, they found only subtle changes in gene expression, throwing the conventional thought of MeCP2 as a transcriptional regulator into question (e.g., Tudor et al., 2002).

In vitro studies have provided the most compelling argument for MeCP2 acting as a transcriptional repressor critical for central nervous system development and function. The picture described in multiple reports is as follows. MeCP2 is initially tightly bound to methylated cytosines within the brain-derived neurotrophic factor (BDNF) promoter. Membrane depolarization triggers calcium-dependent phosphorylation and subsequent release of MeCP2 from its DNA bound state. This releases associated corepressors, allows chromatin remodeling, and permits subsequent activity-dependent transcription to occur (Chen et al., 2003; Martinowich et al., 2003). Using tandem mass spectrometry, phosphospecific antibodies, and elegant biochemical as well as lentiviral assays, the Greenberg lab previously identified a key activity-dependent phosphorylation site of MeCP2 and analyzed its role in nervous system development (Zhou et al., 2006). They demonstrated that neuronal activity drives phosphorylation of MeCP2 at serine 421 in a CamKII-dependent manner specifically in the brain and provided evidence that this single phospho-event is a mediator of activity-dependent dendritic growth, spine maturation and BDNF expression. If S421 phosphorylation relaxes the transcriptional repressor effects of MeCP2 on a select population of activity-dependent genes necessary for nervous system development and function, what are the in vivo effects of blocking this event and how might they contribute to the neurobehavioral abnormalities associated with RTT?

To address these issues, in this study Greenberg and colleagues generated a knock-in mouse in which MeCP2 S421 was mutated to an alanine (MeCP2 S421A) thus preventing activity-dependent phosphorylation of this residue. To investigate the contribution of MeCP2 S421 phosphorylation in cortical circuit formation, the authors examined dendritic morphology of cortical neurons both in vitro and in vivo from MeCP2 S421A mutant animals. Mutant cortical neurons exhibited significantly more dendritic branches and notably, this increase in dendritic complexity was found only in the apical dendritic tufts of pyramidal neurons. This finding however, differs from the reduced spine number and dendritic complexity reported in studies of MeCP2 KO null and RTT patients (Na and Monteggia, 2011). Previous work in MeCP2 null mice showed reduced cortical activity due to a shift in the balance between excitation and inhibition in layer 5 pyramidal neurons. Specifically, reduced circuit excitability was accompanied by both reduced spontaneous excitatory synaptic input and increased inhibition, however the molecular mechanisms which underlie this shift remain largely unknown (Dani et al., 2005). What then, are the neurophysiological consequences of activity-dependent MeCP2 S421 phosphorylation and does this modification influence normal synaptic function and behavior? To address this issue, Cohen et al. (2011) analyzed spontaneous miniature inhibitory postsynaptic currents (mIPSCs) and spontaneous miniature excitatory postsynaptic currents (mEPSCs) in whole-cell recordings from layer II/III pyramidal neurons from MeCP2 S421A mutant animals and control littermates. They observed a modest increase in the amplitude of mIPSCs but no difference in either the amplitude or frequency of mEPSCs. Noting that a hallmark of the early stages of RTT is decreased social function, the authors next examined the behavioral responses of animals in which activity-dependent MeCP2 S421 phosphorylation was abolished. Unlike animals with complete loss of function of MeCP2, MeCP2 S421A animals do not exhibit abnormalities in social interaction, motor coordination, spatial learning, or memory paradigms, but they are unable to distinguish between novel and familiar stimuli. These findings demonstrate a role for activity-dependent phosphorylation of MeCP2 S421 in highly specific and subtle aspects of cortical neuronal morphology, synaptic function, and behaviors.

Adrian Bird and colleagues have challenged the view that MeCP2 functions as a gene-specific transcriptional repressor (Skene et al., 2010). Using a newly developed biochemical fractionation technique, they reported that MeCP2 protein is almost as abundant as the number of histone octamers. They employed bisulfite sequencing and MeCP2 chromatin-immunoprecipitation assays followed by high throughput sequencing (ChIP-Seq) of mouse brain nuclei extract and discovered that MeCP2 is globally distributed across the entire mouse genome and this distribution tracks the density of methyl-CpGs. In the absence of MeCP2, they found elevated levels of histone H3 acetylation (H3Ac), linker histone H1, and transcriptional noise from repetitive elements, but transcriptional levels of specific genes remained unchanged (Skene et al., 2010). These results suggested that high levels of neuronal MeCP2 function to affect global chromatin structure in a genome-wide manner.

One plausible model then is that MeCP2 is bound across the neuronal genome and that activity-dependent phosphorylation of MeCP2 S421 occurs at specific regulatory elements of genes which modulate nervous system development. To address this issue, Cohen and collaborators performed MeCP2 ChIP-Seq with a newly generated pan-MeCP2 antibody and confirmed the observations of Skene et al. that MeCP2 protein is broadly distributed across the neuronal genome with a binding pattern similar to that of histone H3. Next, the authors compared genome binding profiles of MeCP2 before and after neuronal stimulation in neuronal cultures and made the unexpected discovery that MeCP2 remains tightly associated with methylated DNA throughout the neuronal genome regardless of neuronal activation. They also confirmed a similarly widespread pattern of MeCP2 phosphorylation, closely tracking total bound MeCP2 in vivo. If MeCP2 remains constitutively bound to methylated DNA, does MeCP2 S421 phosphorylation effect activity-dependent transcriptional programs? To address this question, the authors employed ChIP-qPCR, ChIP-Seq, and oligonucleotide arrays and, contrary to previous results from in vitro studies, found that induction of activity-dependent genes such as *Bdnf* and *c-fos* remained unchanged regardless of MeCP2 S421 phosphorylation. Furthermore they discovered that this phosphorylation event occurs broadly across the genome in response to

neuronal activation, arguing against a role for MeCP2 S421 phosphorylation as a regulator of activity-dependent gene transcription. These results suggest that MeCP2 functions not as a transcriptional repressor of a specific subset of genes but rather as a core component of chromatin whose activity-induced phosphorylation at a single serine residue controls distinct aspects of nervous system development and function. Aberrations in this process may contribute to the pathophysiology of RTT. Interpretation of the effects of MeCP2 phosphorylation are complicated, however, because phosphorylation occurs at multiple sites which could have different effects on MeCP2 binding and/or activity. A recent study generated a double phosphomutant at S421 and an additional nearby site (S424) and found very different phenotypes, reminiscent of some of the effects of MeCP2 overexpression (Li et al., 2011). This study, like prior studies of MeCP2 phosphorylation, used ChIP at specific promoters and found enhanced occupancy. However Cohen et al. (2011) and Skene et al. (2010) have failed to find selective binding at promoters using ChIP-Seq, raising the possibility of differential sensitivity between these assays.

The present study combines an elegant targeted and specific manipulation in vivo and a detailed profile of MeCP2 binding across the genome, as revealed by ChIP^{-}Seq and suggests a more global view of how tight control of MeCP2 activity must be ensured to control proper brain development. Yet the field as a whole has raised as many questions as it has answered, and an increasingly complex and often discordant view of MeCP2 function is emerging. For example, MeCP2, previously thought to have effects primarily on excitatory synaptic transmission, now appears to play an important role in inhibitory transmission, and knocking out MeCP2 in all inhibitory neurons produces many of the same phenotypes seen in the germline null (Chao et al., 2010). Unlike the phosphomutant, the knockout produces decreased, rather than increased inhibitory input to L2/3 cortical pyramidal neurons. Other studies have suggested that MeCP2 action, initially thought to function primarily in neurons, also has critical functions in glia, and that specific disruption of MeCP2 in glia causes neuronal phenotypes (Lioy et al., 2011). Together, these studies raise the possibility that MeCP2 has both global and local roles. It may play an important part in the maintenance of chromatin integrity in many cell types, but also perform more local functions in regulating subsets of genes in specific cell types. We can only hope that the kind of targeted in vivo manipulation performed by Cohen et al. (2011) coupled to highly discriminating analyses of neuronal and behavioral phenotypes will help resolve this apparent local/global confusion.

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